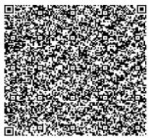

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Nano ZnO and its Perspective in Anti-Cancer Activities

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Abstract

Several methods are used to counter the deadly disease cancer. Zinc oxide nanoparticles (ZnO NPs) is one of the metal oxide nanoparticles which had been used in anti-cancer activities due to its large bandwidth and high exciting binding energy and it has potential applications like antibacterial, antifungal, anti-diabetic, anti-inflammatory, wound healing, antioxidant, optic properties and also which holds promise to treat cancer effectively. Studies have shown that Zinc metal oxide nano particles induce cytotoxicity in cancer cells. The mechanism for antitumor could work through apoptosis or the generation of reactive oxygen species or through necrosis and among other possibilities. This review is on some of the most significant antitumor results obtained with zinc oxide nanoparticles depending on their size, surface morphology, methods of preparation and also its cytotoxicity result.

Keywords: Anti-cancer activity, ZnO nanoparticles, Cancer cell-lines, cytotoxicity

1. Introduction

Cancer is known as one of the most deadly diseases for humankind. According to the NCI Dictionary of Cancer Terms,

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cancer is a term for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems. There are several main types of cancer. Carcinoma is a cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is a cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is a cancer that starts in blood-forming tissue, such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the blood. Lymphoma and multiple myeloma are cancers that begin in the cells of the immune system. Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord. Cancer is also called malignancy. Several methods and ways are implemented to eliminate this disease.

Recently, nanoparticles (NPs) have much attention due to their use in cancer therapy. Studies have shown that different metal oxide nanoparticles induce cytotoxicity in cancer cells, but not in normal cells. In some cases, such anticancer activity has been demonstrated to hold for the nanoparticle alone or in combination with different therapies.[1] Nanomedicine is the current field for biomedical applications, involves engineered nanoparticles to treat the cancer disease and also contains advanced imaging and therapeutic capabilities, for early detection and treatment of various cancer with potentiality.[2] Nanoparticles shows unique properties, so its a preferable choice to replace the conventional treatment. These have biocompatible, active targeting and also passive targeting, and multifunctionality solubility and bioavailability compare to traditional cancer treatments.[3] Nanoparticles bind the drugs and encapsulate drugs to the surface due to their high surface to volume ratio. This leads to higher therapeutic load on to the target and less side effects to the normal cell. The sizes of the nanoparticles are comparable to the biomolecules so they can mimic and hack the activity of biomolecules. Hence, the localized body system, by modification of the surface leads to enhance the small size and also solubility.[2, 3] Nanoparticles as a nanomedicine are now personilized medicines.[4] Several nanoparticles like cerium oxide nanoparticles, Au NPs, Ag NPS,

CuO NPS, ZnO NPs are also used for cancer treatments. The efficacy, the selectivity and the potency in cancer therapy towards the cancer cells show promising result. They show cytotoxicity by zinc-dependent protein activity disequilibrium and ROS induction.[5] This review aims to find the unique properties of ZnO NPs as their role in the selective cytotoxicity to some cancer cell lines of the human body and its dependent of size and shape, and Inhibitory Concentration (IC_{50}) values.

2. ZnO Nanoparticles

The ZnO NPs have received much attention for their applications in cancer therapy. In the group of metal oxides, ZnO NPs are significant for their characterized photocatalytic and photo-oxidizing ability against chemical and biological species. The ZnO nanoparticles are nano-sized particles of ZnO with a size less than 100 nm. They can be prepared by several different methods, such as solid, liquid (i. e., chemical) and gaseous. There are a variety of chemical methods, for example mechanochemical process, precipitation process, precipitation in the presence of surfactant, sol-gel method, solvo-thermal, hydrothermal, emulsion and micro-emulsion methods.[6] Surfactant, polymer molecules, or, Triton-X 100 or PEG are used as a stabilising agents. The main significance of nanoparticles is that the size reduction to nanoscale that leads to the development of new unique physicochemical, structural, electronic and magnetic properties of nanoparticles, which are not present in their macro or bulkier form.[7] A variety of ZnO nanostructures have been synthesized, including nanoparticles, nanowires, nanorods, nanotubes, nanobelts and other complex morphologies. They have wide range of applications in cancer therapy, biosensing, drug/gene delivery, nanomachines that can act as biological mimetic, biomaterials for tissue engineering, shape memory polymers such as molecular switches, etc.[8]

Nowadays, ZnO NPs have drawn attention for their effectiveness in cancer therapy.[6] Studies have shown that ZnO NPs induce cytotoxicity selectively in a cell-specific and proliferation-dependent manner.[9–11] The cytotoxicity of ZnO NPs toward

mammalian cells, and other types of cells are to be considered. The anticancer activity of ZnO NPs, mechanisms of apoptosis in cancer cells due to ZnO NP treatment, etc. are still to be worked.

The nanoparticles of zinc oxide were synthesized by the facile precipitation method with starch as the nontoxic capping agent. The average crystallite size was estimated from the peak width at half maximum using Scherer formula and the calculated crystallites size is 32.7 nm. To further support the formation mechanism of ZnO NPs, FTIR studies were performed. The fundamental mode of vibration at 3404.13 cm^{-1} corresponds to the O-H stretching vibration due to the water molecule present in the sample. The peaks at 2928.23 cm^{-1} and 1007.06 cm^{-1} are due to the presence of C-O and C-H stretching vibrations indicating the saccharide strucuture of starch. The peak at 759.92 cm^{-1} is attributed to the C-O bond stretching. The band at 1415.77 cm^{-1} and 1644.52 cm^{-1} correspond to the C=O asymmetric and the C=O symmetric stretching vibration. The prominent peaks at 702.33 cm^{-1} and 521.57 cm^{-1} indicate the stretching vibrations of ZnO nanoparticle.

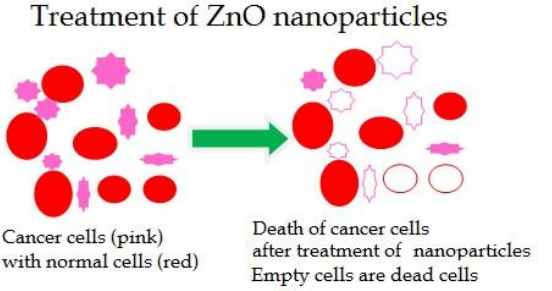

Figure 1. Pictorial representation of cytotoxicity of ZnO NPs on different cancer cells

The average crystallite size is 32.7 nm having rice like morphology. The MTT assay is used to for synthesized ZnO NPs, have been tested for their efficacy of cytotoxic activity against MCF-7 (breast cancer cells). The cell viability evaluated after 24 h exposure to ZnO NPs of various concentrations ranging from (10 µg/ml- 200 µg/ml). The ZnO NPs induced cytotoxicity on MCF-7 cell lines was found to be increasing with an increase in concentration of ZnO NPs. The 50% viability happens at the concentration of 40 µg/ml which is the half maximal. The IC_{50} and the metabolic activity are

characteristic, which decrease with the decreasing of the dose of ZnO NPs incubated with the MCF-7cells.[12]

The zinc oxide NPs were used at a very low concentration and were found to exhibit activity against HepG2 (liver cancer) and MCF-7 (breast cancer) cancer cells in a dose-dependent manner: viability, measured by the MTT assay, showed a dose-dependent decrease. At a very low concentration such as 25 µg/ml the cell viability was less than 10% in the case of HepG2 cells. The results of emphasis on the anti-proliferative studies clearly demonstrate that treatments with NPs sensitize cancer cells. The degree of apoptosis was found to be enhanced with an increase in the concentration of NPs, and a significant concentration of NPs resulted in cell death in both cancer cell lines.[13] In that study, quantitative real-time PCR was utilized to analyze the mRNA levels of apoptotic markers (p53, Bax, bcl-2 and caspase-3) in HepG2 cells exposed to ZnO NPs at a concentration of 50 µg/ml for 24 h. The results showed that the mRNA levels of these apoptotic markers were significantly altered in HepG2 cells due to ZnO NP exposure. The mRNA level of the tumor suppression gene p53 was 1.9-fold higher and the mRNA expression levels of the pro-apoptotic gene Bax and the anti-apoptotic gene bcl-2 were decreased (2.7- and 2.5-fold, respectively) in the exposed cells, compared to untreated cells. Moreover, the effect of ZnO NPs on the mRNA expression level of caspase-3 was studied and was found to be 1.8-fold higher in the treated cells than the untreated control cells. The mRNA expression levels of p53, Bax, bcl-2 and caspase in HepG2 cells in response to ZnO NP exposure were studied because apoptosis is controlled through these pathways. The quantitative real-time PCR results show that ZnO NPs up-regulate the mRNA levels of the cell cycle checkpoint protein p53 and the pro-apoptotic protein Bax. The expression of the anti-apoptotic protein bcl-2 was down-regulated in cells exposed to ZnO NPs. Furthermore, the up-regulation of p53 and the down-regulation of bcl-2 family members, such as Bax, induce the permeabilization of the outer mitochondrial membrane, which releases soluble proteins from the intermembrane space into the cytosol, where they promote caspase activation.[14]

Wahab *et al.* have synthesized ZnO nanoparticle with the average size of approximately 10 nm, characterized, studied morphology and their induction of oxidative stress in Cloudman S91 melanoma cancer cells was studied.[15] Various doses of ZnO nanoparticles were treated with melanoma cancer cells for 24 h of incubation at 37ºC. MTT assay was done for the cell viability whereas the morphology of the cells was observed via confocal laser scanning microscopy (CLSM), which revealed that when the time interval was increased, the number of cells decreased. The apoptosis-correlated, intracellular production of reactive oxygen species (ROS) was also measured with melanoma cancer cells with varying ZnO nanoparticle doses. After pre-incubation with ZnO N Ps in concentrations of 0.002-20 µg/ml, the HNSCC cell lines HLaC 78 and UD-SCC 7A as well as primary oral mucosa cells (pOMCs) were treated with UVA-1. Cell survival and viability was observed by 3-(4, 5-dimethylthiazol-2-yl)-2,5- diphenyl-tetrazoliumbromide-(MTT)-assay and fluorescein diacetate test. Apoptosis was assessed by annexin-V propidium iodide flow cytometry. Intranuclear distribution of the rod-shaped particles was observed in 3.5% of head and neck squamous cell carcinoma cell lines (HNSCC) and in 0.5% of pOMCs. UVA-1 irradiation for 15 min in combination with 0.2 and 2 µg/ml of ZnO NP dispersion was shown to reduce the viability of cancer cell lines significantly in comparison to cells without NP exposure or UVA-1 treatment only. For HLaC 78, a significant reduction in viable cells was seen at 10 min of UVA-1 treatment and a ZnO NP concentration of 2 µg/ml. For HLaC 78, a significant reduction in viable cells was seen at 10 min of UVA-1 treatment and a ZnO NP concentration of 2 µg/ml. Flow cytometry indicated that cell death occurred primarily through necrosis. In pOMCs, vitality was not influenced either by UVA-1 treatment orZnO NP exposure up to 2 µg/ml or a combination of both.ZnO NPs showed cytotoxicity at 20 µg/ml without UVA-1. Due to their photocatalytic properties, ZnO NPs may induce cell death in human HNSCC cell lines in vitro. Further studies will evaluate a possible benefit in adjuvant cancer therapy [15]. Hackenbert *et al.* synthesized ZnO NP of particle size 74nm pre-incubation followed by UVA-1 irradiation induced a significant reduction in viable HNSCC in vitro [16].

Therapeutic cancer vaccines are emerging as part of an anticancer regimen that utilizes specific antigens to initiate and modulate the antitumor immune response.[17] Dendritic cells (DCs) have been used for therapeutic cancer vaccines.[18] DC-based cancer immunotherapy that destroys tumors requires a clinically-suitable delivery system for the target antigens in the DCs. In one study, the authors developed iron oxide (Fe_3O_4)-zinc oxide (ZnO) core-shell NPs (CSNPs) to have ZnO-binding peptides to carry tumor antigens in DCs. This NP-antigen complex was efficiently taken up by the DCs and was demonstrated to function as a cancer immunotherapy via injection of the DCs containing the CSNP-antigen complex into the hind footpads of mice. Mice immunized with DCs containing the NP-antigen complex showed enhanced tumor antigen-specific T-cell responses, delayed tumour growth and better survival than controls.[19] However, a study of the potential toxicity of these new nanocarriers is required. Recently, a repeated toxicity study was performed *in vivo* by subcutaneous injection in mice and showed a dose-dependent increase in granulomatous inflammation at the injection site of the CSNP-treated animals. But no alterations in the body and other histopathological lesions in other organs could be attributed to the CSNPs.[20]

In another study, highly pure ZnO nanoparticles with a narrow size distribution of 16-19 nm prepared by the simple DMC (Dry Mechano-Chemical) method are considered. The anticancer activity on MCF-7 (Breast cancer cell) and A549 (Lung Cancer cell) were determined by the MTT (Methylthiazolydiphenyl-tetrazolium bromide) assay. A549 and MCF-7 cells were exposed to ZnO-NPs and they exhibited 50% reduction at a very low concentration 31.2 µg/ml. Thus, the reduction in cell viability with NPs induces cytotoxicity in cancerous cells. There is a size dependent effectiveness of ZnO nanoparticles in the removal of cancer cells and also a positive correlation with reduced toxicity. ZnO nanoparticles with an average size between 16 to 19 nm and sphere shapes were successfully synthesized using dry grinding method. The synthesis method reported here is easily scalable for large scale production, economically feasible, biocompatible and cost effective.

The results suggest that with the aid of oxide based nanoparticles conditional chemotherapeutic agents may have even broader range of applications in the treatment of cancer cells. The dosage particles' size-dependent activity against cancer cells and the variation in toxicity need to be further investigated to establish optimum standards.[21]

3. Conclusion

This review is on ZnO nanoparticles. Zinc oxide NPs' role in the human body as anti-cancer agent depends on the size, dosage, the surface morphology and IC_{50} value. The probable mechanism surrounding ZnO NPs with the biology of the human body, leads to its selective localization and cytotoxicity towards cancer cells. While ZnO NPs induce cytotoxicity towards cancer cells through oxidative stress via ROS generation, the response of zinc-mediated protein activity disequilibrium as a result of high levels of intracellular zinc ions is a more likely cause. There is *in vivo* research into nanoparticles for design of NPs for their better clinical use. A society-oriented collaboration leads to the development of smart nanoparticles with accurcy of selectivity and toxicity towards cancer cells with biocompatibility.

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